

**DATA EVALUATION RECORD  
MYSID CHRONIC TOXICITY TEST  
GUIDELINE OPPTS 850.1350**

1. **CHEMICAL**: Metconazole

**PC Code**: 125619

2. **TEST MATERIAL**: Metconazole

**Purity**: 99.7%

3. **CITATION**

**Author**: Lee, M.R.

**Title**: Metconazole (KNF-S-474m) – Life-Cycle Toxicity Test with  
Mysids (*Americamysis bahia*)

**Study Completion Date**: August 17, 2010

**Laboratory**: Springborn Smithers Laboratories  
790 Main Street  
Wareham, MA 02571-1037

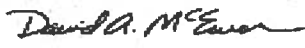
**Sponsor**: Valent USA Corporation  
6560 Trinity Court  
Dublin, CA 94568

**Laboratory Report ID**: 12709.6272

**MRID**: 48221501

**DP Barcode**: 387425

4. **REVIEWED BY**: David A. McEwen, Staff Scientist, CSS-Dynamac Corporation

**Signature**: 

**Date**: 7/23/13

**APPROVED BY**: Teri S. Myers, Environmental Scientist, CDM Smith

**Signature**: 

**Date**: 8/12/13

5. **REVIEWED BY**: Lewis R. Brown, Environmental Biologist

**Signature**: 

**Date**: 9/12/13

Michael Lowit, Ph.D., Ecologist

**Signature**: 

**Date**: 11/6/13

## 6. STUDY PARAMETERS

<b>Age of Test Organism:</b>	Neonates, 21 hours old
<b>Definitive Test Duration:</b>	28 days
<b>Study Method:</b>	Flow-through
<b>Type of Concentrations:</b>	Mean-measured

## 7. CONCLUSIONS

NOAEC = 59 µg ai/L

LOAEC > 59 µg ai/L

Endpoint(s) Affected: none

## 8. ADEQUACY OF THE STUDY

**A. Classification:** ACCEPTABLE

**B. Rationale:** N/A

**C. Repairability:** N/A

## 9. MAJOR GUIDELINE DEVIATIONS

1. The photoperiod (16 hr light:8 hr dark) deviated slightly from recommendations (14 hr light:10 hr dark).
2. The body length of each mysid was not recorded at the time of sexual discernment.
3. The time to first brood release was not assessed by the study author as a toxicological endpoint; however, replicate data were provided for the reviewer to verify effects on this endpoint.
4. The mean number of offspring in the control (20/female or ca. 1.5/female/day) was lower than criteria in the 1996 draft 850.1350 guideline (3/female/day).

Theses deviations do not affect the acceptability of the study.

**10. MATERIALS AND METHODS****A. Biological System**

Guideline Criteria	Reported Information
<b>Species:</b> An estuarine shrimp species, preferably <i>Americamysis bahia</i> .	<i>Americamysis bahia</i>
<b>Duration of the Test:</b> 28 days	28 days
<b>Source (or supplier):</b> Should originate from in-house cultures.	In-house cultures maintained for 2 months. The brood stock was originally obtained from the U.S. EPA Atlantic Ecology Division Laboratory (Narragansett, RI) in May 2009.
<b>Parental Acclimation:</b> Within a 24-hr period, changes in water temperature should not exceed 1°C, while salinity changes should not exceed 5%.  Mysids should be in good health.	Mysids were cultured in six re-circulating 80-L glass aquaria in dilute, natural seawater (same as that used during the definitive study). During the 14-day period prior to testing, the seawater was characterized as having a salinity of 22 to 26‰, pH of 7.9 to 8.0, and dissolved oxygen of 6.8 to 7.0 mg/L. The cultures were maintained under a 16-hr light (810 lux)/8-hr dark photoperiod at 22 to 26°C. Feeding was not described. It was reported that the culture organisms did not show any sign of sickness, disease, injuries, or abnormalities, and that the brood stock appeared to be in good health at test initiation.

Guideline Criteria	Reported Information
<b>Chamber Location:</b> Treatments should be randomly assigned to test chamber locations.	Organisms were impartially selected and distributed to test compartments. Treatment vessel assignments with respect to location to each other were not reported.
<b>Distribution:</b> Minimum of 40 mysids per concentration  Mysids should be separated into replicate groups of no more than eight individuals when most of the mysids reach sexual maturity (usually 10 to 14 days after test initiation).	60/level prior to pairing: 15 mysids per retention chamber, two retention chambers per aquarium, and two replicate aquaria per treatment level.  At maturity, ten isolated male/female pairs per replicate aquarium (20 pairs/level); excess organisms were maintained in one of the initial retention chambers within the replicate until they were paired or test termination. Male mysids from the pooled excess organisms were used to replace dead males from the paired groups; females were not replaced.
<b>Pairing:</b> Should be conducted when most of the mysids are sexually mature (usually 10-14 days after test initiation)	Mysids were paired on day 13 (when sexual maturity was reached based on the appearance of gravid females).
<b>Offspring Exposure:</b> Live young must be counted and separated into retention chambers at the same concentration where they originated.	Groups of offspring (10 per replicate, 20 per treatment level) were placed in separate pairing chambers within the replicate and maintained for 96 hours.
<b>Observations:</b>	<ul style="list-style-type: none"> <li>- Adult mysids were observed daily for mortality, sub-lethal effects, and reproduction (post-pairing).</li> <li>- Offspring were observed daily for mortality and sub-lethal effects.</li> </ul>

Guideline Criteria	Reported Information
<p><b>Feeding:</b> Mysids should be fed during testing. A recommended food is live <i>Artemia</i> spp. Nauplii (ca. 48-hr old).</p>	<p>Mysids were fed live brine shrimp (<i>Artemia salina</i>) nauplii, <math>\leq 48</math> hrs old, twice daily. At least one of these feedings was with brine shrimp nauplii enriched with Selco® (a supplemental substance high in saturated fatty acids).</p> <p><u>F<sub>0</sub>-generation</u>  Days 0-4: 120 nauplii/mysid  Days 5-7: 180 nauplii/mysid  Days 8-9: 240 nauplii/mysid  Days 10-12: 300 nauplii/mysid  Days 13+ (pairing chambers): 450 nauplii/mysid  Days 13+ (retention chambers): 3600 nauplii/chamber</p> <p><u>F<sub>1</sub>-generation</u>  120 nauplii/mysid</p>
<p><b>Controls:</b> Negative control and carrier control (when applicable) are required.</p>	<p>A negative control group was included.</p>

### Comments

Excess brine shrimp and organic debris in the test chambers were removed daily when observed.

The maximum organism loading concentration (based on a conservative wet weight of 0.0045 g per adult mysid) was 0.0026 g of biomass/L of flowing test solution per day.

The study was initiated on June 2, 2008, and the in-life phase of the definitive test was conducted from January 27, 2010 to February 24, 2010.

**B. Physical System**

Guideline Criteria	Reported Information
<p><b>Test Item:</b></p>	<p><u>Metconazole Technical</u>  Synonyms: KNF-S-474m  IUPAC name: (1RS,5RS;1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol  CAS name: 5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol  CAS no.: 125116-23-6  Description: Not reported  Lot no.: AS2122a  Purity: 99.7% (84.6% <i>cis</i> isomer and 15.1% <i>trans</i> isomer)  Storage: Frozen (-10 to -25°C)</p>
<p><b>Test Water:</b>  May be natural or artificial seawater.</p> <p>Natural seawater should be filtered (&gt;20 µm).</p> <p>Artificial seawater should be prepared with deionized (conductivity &lt;0.1 mS/M at 12°C) or glass-distilled water. When deionized water is prepared from a natural water source, conductivity and TOC (or COD) should be measured in each batch.</p>	<p>Dilution water consisted of diluted, filtered natural seawater. The seawater was pumped from the Cape Cod Canal (Bourne, MA) from <i>ca.</i> 1- 4-m offshore at a depth of <i>ca.</i> 0.5 m. In the laboratory, the seawater was adjusted to a salinity of 20 ± 3‰ with laboratory well water, filtered (20- and 5-µm) and re-circulated prior to use.</p> <p>The seawater used for this study had a salinity range of 20 to 22‰ and pH range of 6.9 to 8.7. The TOC of the dilution water ranged from 1.0 to 1.5 mg/L during the definitive test.</p> <p>Results of periodic analysis for pesticides, organics, and metals indicated that none of these compounds were detected at concentrations that are considered toxic in any of the water samples analyzed.</p>

Guideline Criteria	Reported Information
<p><b>Salinity:</b>  <math>20 \pm 3\text{‰}</math> (parts per thousand).</p> <p>Should be measured weekly in each chamber.</p>	<p>21 to 23‰</p> <p>Measured in all replicates on day 0, and in alternating replicates from each level daily thereafter.</p>
<p><b>pH:</b>  Should be measured weekly in each chamber.</p>	<p>7.4 to 7.9</p> <p>Measured in all replicates on day 0, and in alternating replicates from each level daily thereafter.</p>
<p><b>Dissolved oxygen:</b>  Should remain between 60 and 105% saturation.</p> <p>Should be measured weekly in each chamber.</p>	<p>77 to 113% saturation</p> <p>Measured in all replicates on day 0, and in alternating replicates from each level daily thereafter.</p>
<p><b>Test Temperature:</b>  <math>25 \pm 2^{\circ}\text{C}</math></p> <p>Should be measured weekly in each chamber.</p>	<p>Daily: 25 to 29°C (see Comments below)  Continuous: 25 to 28°C</p> <p>Measured in all replicates on day 0, and in alternating replicates from each level daily thereafter. In addition, temperature was continuously monitored in one control replicate.</p>
<p><b>Photoperiod:</b>  14 hr light/10 hr dark with 15- to 30-minute transition periods</p>	<p>16-hour light, 8-hour dark photoperiod, with 30-minute transition periods.</p> <p>Intensity ranged from 210 to 240 lux.</p>

Guideline Criteria	Reported Information
<p><b>Dosing Apparatus:</b></p> <p>(1) Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p> <p>(2) A minimum of 5 toxicant concentrations</p> <p>(3) A dilution factor not greater than 0.5 and controls should be used.</p>	<p>(1) Intermittent-flow proportional diluter</p> <p>(2) Six toxicant concentrations</p> <p>(3) A dilution factor of 0.5 plus a negative control was used.</p>
<p><b>Flow Rate:</b></p> <p>(1) Flow rates should provide <math>\geq 5</math> volume additions per 24 hr.</p> <p>(2) Flow splitting accuracy must be within 10%.</p> <p>(3) Meter systems calibrated before study and general operation checked twice daily during test period.</p>	<p>(1) 7.4 volume additions/day, equivalent to a 90% test solution replacement rate of <i>ca.</i> 7 hours.</p> <p>(2) Flow splitting accuracy was reported to be within 5%.</p> <p>(3) The FMI pump was calibrated before the study. The function of the diluter system was monitored daily and a visual check was performed twice daily.</p>
<p><b>Test Vessels:</b></p> <p>Materials and equipment that minimize sorption. Test vessels should be loosely covered.</p> <p><b>Retention Chambers:</b></p> <p>Can be constructed with netting material of appropriate mesh size.</p>	<p>Glass aquaria, measuring 39 x 20 x 25 cm with a 9-cm side drain. It was not reported if test chambers were covered.</p> <p>Prior to pairing: glass Petri dishes, 10-cm diameter, 2-cm deep, to which a 15-cm high Nitex® screen collar (250-<math>\mu</math>m mesh size) was attached. The solution volume within each retention chamber was 390 to 710 mL.</p> <p>Following pairing: 6-cm diameter Petri dishes, to which a 13-cm high Nitex® screen collar (250-<math>\mu</math>m mesh size) was attached. The solution volume within each pairing chamber was 140 to 250 mL.</p>
<p><b>Aeration:</b></p> <p>Permitted if necessary to maintain DO.</p>	<p>None reported.</p>



Comments

The exposure system was in proper operation for 9 days prior to test initiation to allow for equilibration.

On days 0 and 1, the daily temperature measurements were above the recommended range. No abnormal behavior was observed in any organism during this period or at any time following and it was concluded that the deviation had no impact on the results of the study.

**C. Chemical System**

Guideline Criteria	Reported Information
<b>Concentrations:</b> Concentration ranges should be selected to determine the concentration response curves, LC <sub>50</sub> values, and MATC.  Toxicant level should be measured at each level at 0, 7, 14, 21, and 28 days, and should not vary more than 20% among replicate test chambers.	Nominal: negative control, 1.9, 3.8, 7.5, 15, 30, and 60 µg ai/L  Mean-measured: <0.46 (<LOQ, control), 1.9, 4.0, 7.7, 16, 30, and 59 µg ai/L  Water samples were collected from alternate replicate vessels (all levels) on days 0, 7, 14, 21, and 28.  Mean-measured concentrations ranged from 98 to 110% of nominal.
<b>Solvents:</b> Should not exceed 0.1 ml/L in a flow-through system.  Acceptable solvents: triethylene glycol, methanol, acetone, and ethanol.	N/A

Comments

The nominal concentrations for the definitive study were based upon consultation with the Sponsor.

A 300 µg ai/mL primary stock solution was prepared prior to exposure initiation and every other day thereafter by the addition of 0.0301 g of metconazole (0.0300 g ai) to 2 L of deionized water. The solution was sonicated in a warm water bath for *ca.* 30 minutes. Following sonication, the solution was diluted to 100 L with dilution water and mixed for *ca.* 48 hours. The primary stock solutions were clear and colorless following mixing.

Three quality control (QC) samples were prepared at each sampling interval at nominal concentrations of 0.900, 10.0, and 60.0 µg ai/L and remained with the set of exposure solution samples throughout the analytical process. Recoveries from QC samples ranged from 86.6 to 113% of nominal concentrations (n=15).

## 11. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was performed according to U.S. EPA Good Laboratory Practice Standards (40 CFR, Part 160) with the following exceptions: routine non-GLP food and water screening analyses. It was reported, however, that the analyses were conducted following standard validated methods.
<p><b>Controls:</b></p> <p>(1) Survival of the first-generation controls (between pairing and test termination) must not be less than 70%.</p> <p>(2) At least 75% of the paired 1<sup>st</sup> generation females in the controls produced young or</p> <p>(3) The average number of total young produced by the 1<sup>st</sup> generation females in the control(s) was at least 3. Note that the 1996 DRAFT 850.1350 guideline criteria is 3 offspring/female/day.</p>	<p>(1) The average post-pairing control survival was 93% (according to the study author's calculations, adjusted for non-toxicant related death). The average post-pairing control survival calculated by the reviewer, including all mortalities, was 75% (post pairing survival was 20/29 and 24/30 in replicates A and B, respectively). One mysid in replicate A was inadvertently killed during cleaning and was excluded from the preceding calculations.</p> <p>(2) &amp; (3) 95% of paired negative control females produced an average of 20 offspring per female (<i>ca.</i> 1.5/female/day).</p>

Guideline Criteria	Reported Information
<p><b>Data Endpoints</b> must include:</p> <ul style="list-style-type: none"> <li>(1) The number of dead adult mysids on days 7, 14, 21, and 28. Concentration-response curves, LC<sub>50</sub> values, and associated 95% C.I. for each interval.</li> <li>(2) Body length of male and female mysids at the time of sexual discernment and again on day 28.</li> <li>(3) Time to first brood release.</li> <li>(4) Cumulative young per female.</li> <li>(5) If available, mortality, number of each sex, and body lengths of each sex should be recorded for the offspring.</li> <li>(6) Any abnormal behavior or appearance</li> </ul>	<p><b>Endpoints evaluated in this study included:</b></p> <ul style="list-style-type: none"> <li>(1) Survival of male, female, and total adult mysids on day 28.</li> <li>(2) Body lengths and dry weights of male and female mysids at day 28.</li> <li>(3) Not evaluated</li> <li>(4) Number of offspring per female</li> <li>(5) 96-hour survival of offspring</li> <li>(6) Any abnormal behavior or appearance</li> </ul>
<b>Raw data included?</b>	Yes

**A. Effects Data**Adult (F<sub>0</sub>)

Toxicant Conc. (µg ai/L)		Time to First Brood Release (days)	Mean No. Offspring/ Female	Day 28 <sup>(a)</sup>						
				Mean Survival (%) <sup>(c)</sup>			Mean Total Length (mm)		Mean Dry Weight (mg)	
Nominal	Measured			Day 28	♂	♀	♂	♀	♂	♀
Control	<LOO <sup>(b)</sup>	18	20	93	86	100	6.9	7.4	0.80	1.03
1.9	1.9	17.5	22	78	84	85	7.0	7.5	0.78	1.01
3.8	4.0	18	20	89	100	90	7.0	7.3	0.70	1.05
7.5	7.7	18	18	85	96	90	7.0	7.3	0.79	1.10
15	16	19	20	91	100	93	7.1	7.5	0.81	1.16
30	30	18.5	16	85	85	94	7.0	7.3	0.81	0.98
60	59	19	20	82	87	92	6.9	7.4	0.80	1.08

<sup>(a)</sup> Data were obtained from Tables 3-6 on pages 35-38 of the study report. Rounded values are reported. Values for length and weight reflect the mean of all test organisms alive at test termination (paired and unpaired).

<sup>(b)</sup> LOQ = 0.38 to 0.46 µg ai/L

<sup>(c)</sup> Survival data reported here are the percentages calculated by the study author. The study author omitted the deaths of certain individuals that were claimed to be non-toxicant related (e.g. impingement). The reviewer did not correct survival percentages for accidental deaths, as it was unclear which deaths were and were not treatment-related, and therefore the survival data of the study author and reviewer differ. The survival percentages at day 28 analyzed by the study author (not separated by sex) were 73, 65, 73, 67, 83, 77, and 70% for the negative control and mean measured exposure concentrations of 1.9, 4.0, 7.7, 16, 30, and 59 µg ai/L, respectively.

### Offspring (F<sub>1</sub>)

Toxicant Concentration (µg ai/L)		Mean Survival (%) after 96 Hours <sup>(a)</sup>
Nominal	Mean Measured	
Control	<LOQ <sup>(b)</sup>	100
1.9	1.9	100
3.8	4.0	100
7.5	7.7	100
15	16	100
30	30	100
60	59	100

<sup>(a)</sup> Data were obtained from Table 7 on page 39 of the study report.

<sup>(b)</sup> LOQ = 0.38 to 0.46 µg ai/L

Based upon visual assessment of the data, no treatment-related effect was observed on the 96-hour post-release survival of offspring at any treatment level, which averaged 100% for all levels. The reported NOAEC and LOAEC values for offspring survival were 59 and >59 µg ai/L, respectively.

### **B. Toxicity Observations**

All (control-level) validity requirements were met: >70% survival of first-generation mysids between pairing and test termination, >75% of the females in the control released young, and the controls produced >3 offspring per female total (note that the 1996 DRAFT 850.1350 guideline criteria is 3 offspring/female/day).

No treatment-related effects were observed on overall survival, or on post-pairing male or female survival. For all control and treatment levels, overall survival ranged from 78 to 93%, post-pairing male survival ranged from 84 to 100%, and post-pairing female survival ranged from 85 to 100%. The reported LC<sub>50</sub> was >59 µg ai/L. The reported NOAEC and LOAEC were 59 and >59 µg ai/L, respectively.

The time to first brood release was not evaluated as a toxicological endpoint. Reviewer-calculated times to mean brood release were 18, 17.5, 18, 18, 19, 18.5 and 19 days for the control and mean-measured 1.9, 4.0, 7.7, 16, 30, and 59  $\mu\text{g ai/L}$  treatment levels, respectively (see section 14). Based upon visual inspection of the data, there was no treatment-related effect on the time to first brood release.

For all levels, 95 to 100% of paired females produced young. The number of offspring per female averaged 20 for the negative control group and 22, 20, 18, 20, 16, and 20 for the mean-measured 1.9, 4.0, 7.7, 16, 30, and 59  $\mu\text{g ai/L}$  levels, respectively. No statistically-significant differences from controls were observed at any treatment level. The reported NOAEC and LOAEC values for reproduction were 59 and  $>59 \mu\text{g ai/L}$ , respectively.

No clinical signs of toxicity were reported.

Gender-specific growth was assessed as total body length and dry weight at study termination. In males, total body lengths ranged from 6.9 to 7.1 mm and dry body weights ranged from 0.70 to 0.81 mg for all levels (including the control), with no statistically-significant differences indicated at any level compared to the control for either parameter. In females, total body lengths ranged from 7.3 to 7.5 mm and dry body weights ranged from 0.98 to 1.16 mg for all levels (including the control), with no statistically-significant differences indicated at any level compared to the control for either parameter. The reported NOAEC and LOAEC values for growth were 59 and  $>59 \mu\text{g ai/L}$ , respectively.

### **C. Statistical Results**

Endpoints that were statistically-analyzed included 28-day survival, male and female post-pairing survival, growth of males and females (dry body weight and total length), and reproduction (number of young release per female). Survival of offspring was visually-assessed for treatment-related effects.

Data were checked for normality using the Shapiro-Wilk's Test and for homogeneity of variance using Bartlett's Test. The 28-day, male and female survival data failed the assumptions for homogeneity. All survival data were subsequently analyzed using the non-parametric Kruskal-Wallis' Test. Remaining data were analyzed using ANOVA and Dunnett's Test. All statistical conclusions were made at the 95% level of certainty except in the basic assumption tests (e.g., Shapiro-Wilk's Test and Bartlett's Test), in which the 99% level of certainty was applied.

The NOAEC and LOAEC were based on significance data, and the MATC was calculated as the geometric mean of these limits. All analyses were performed using TOXSTAT® Version 3.5 (1996) statistical software and mean-measured concentrations.

During this study, no concentration tested caused a reduction of 50% survival, therefore, the LC<sub>50</sub> value was empirically estimated to be greater than the highest mean-measured concentration tested and no statistical analyses were performed.

Endpoint	Method	NOAEC (µg ai/L)	LOAEC (µg ai/L)	MATC (µg ai/L)
F <sub>0</sub> Survival (28 days)	Kruskal-Wallis	59	>59	N/A
F <sub>0</sub> Survival, Male	Kruskal-Wallis	59	>59	N/A
F <sub>0</sub> Survival, Female	Kruskal-Wallis	59	>59	N/A
Offspring/Female	Dunnett's	59	>59	N/A
Total Length, Male	Dunnett's	59	>59	N/A
Total Length, Female	Dunnett's	59	>59	N/A
Dry Weight, Male	Dunnett's	59	>59	N/A
Dry Weight, Female	Dunnett's	59	>59	N/A
F <sub>1</sub> Survival (96 hr)	Visually assessed	59	>59	N/A

## 12. REVIEWER'S STATISTICAL RESULTS

The reviewer analyzed the endpoints for F<sub>0</sub> survival, offspring/female, total length of males and females, dry weight of males and females, and F<sub>1</sub> survival. All statistical analyses were performed using CETIS version 1.8.7.7 statistical software with backend database settings implemented by EFED on 29 May 2013. For the survival endpoints, all mortalities were counted, regardless of cause, as it was unclear whether deaths were treatment-related (the study author noted many mysids as missing, impinged, or accidentally killed during cleaning). All males and females (paired and unpaired) were included for analysis of growth measurements (length and body weight). The male and female weight data were confirmed to be normally distributed and have homogeneous variances using Shapiro-Wilk's and Bartlett's tests, respectively. Female length and offspring/female data were found to be normally distributed using Shapiro-Wilk's tests, but no homogeneity of variance tests were run for these parameters. Male length was not normally distributed according to the Shapiro-Wilk's test (homogeneity of variance was not tested). The F<sub>0</sub> survival data were confirmed to be normally distributed using Shapiro-Wilk's tests, but was found to have unequal variances using Levene's test. The time to first brood data was found to have a non-normal distribution using Shapiro-Wilk's

test but was not assessed for homogeneity of variance. The EFED Statistics Reference Guide to Ecotoxicity Studies (v1.3.0) states that normality should be assumed and a parametric test used when  $n=2$ ; therefore, statistical analyses are based on Dunnett's test (generally there were no clear trends in the data). The F1 survival data were visually assessed. All analyses were based on mean-measured exposure concentrations.

Endpoint	Method	NOAEC ( $\mu\text{g ai/L}$ )	LOAEC ( $\mu\text{g ai/L}$ )
F0 Survival (28 days)	Dunnett's	59	>59
Offspring/female	Dunnett's	59	>59
Time to First Brood	Dunnett's	59	>59
Total Length, Males	Dunnett's	59	>59
Total Length, Females	Dunnett's	59	>59
Dry Weight, Males	Dunnett's	59	>59
Dry Weight, Females	Dunnett's	59	>59
F1 survival (96 hours)	Visual assessment	59	>59

#### General Comments

The study author noted in Appendix 3 of the study report that many mysids were missing, impinged, or accidentally killed during cleaning. These mortalities were included in the reviewer's survival calculations as it was unclear which deaths were treatment-related. Despite the differences between the reviewer and study author's survival calculations, the overall conclusions were the same. There was no toxicity observed in this test.

In replicate B at the 30  $\mu\text{g ai/L}$  level, the table on page 82 states that there were 27 mysids after 11 days (22 live males/immature mysids plus 5 live females). After 13 days, 28 mysids were alive (18 live males/immature mysids plus 10 live females). It is unclear how the number of mysids increased between days 11 and 13. The reviewer entered the survival data as 28 mysids at pairing (day 13) in CETIS.



### 13. REFERENCES

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**14. COPY OF REVIEWER'S MEAN TIME TO FIRST BROOD RELEASE CALCULATIONS**

Mean-Measured Concentration ( $\mu\text{g ai/L}$ )	Replicate	Day of First Brood Release
Control	A	18
	B	18
	<b>Mean</b>	18
1.9	A	18
	B	17
	<b>Mean</b>	17.5
4	A	18
	B	18
	<b>Mean</b>	18
7.7	A	18
	B	18
	<b>Mean</b>	18
16	A	19
	B	19
	<b>Mean</b>	19
30	A	18
	B	19
	<b>Mean</b>	18.5
59	A	19
	B	19
	<b>Mean</b>	19

# CETIS Summary Report

Report Date: 02 Jul-13 10:49 (p 1 of 5)  
 Test Code: 125619 48221501 | 16-2078-2964

OPPTS 850.1350 Chronic Invert (Mysid)				Springborn Smithers	
<b>Batch ID:</b>	18-8035-0752	<b>Test Type:</b>	Chronic Mysid (28-d)	<b>Analyst:</b>	
<b>Start Date:</b>	27 Jan-10	<b>Protocol:</b>	OPPTS 850.1350 Chronic Invert (Mysid Life	<b>Diluent:</b>	Seawater
<b>Ending Date:</b>	24 Feb-10	<b>Species:</b>	Americamysis bahia	<b>Brine:</b>	
<b>Duration:</b>	28d 0h	<b>Source:</b>	Lab In-House Culture	<b>Age:</b>	21 h
<b>Sample ID:</b>	17-4264-5991	<b>Code:</b>	48221501	<b>Client:</b>	CDM Smith
<b>Sample Date:</b>	27 Jan-10	<b>Material:</b>	Metconazole	<b>Project:</b>	Unknown
<b>Receive Date:</b>		<b>Source:</b>	Valent U.S.A. Corporation		
<b>Sample Age:</b>	NA	<b>Station:</b>			
<b>Batch Note:</b>	125619 48221501 flow-through				
<b>Sample Note:</b>	125619 48221501 flow-through				

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-1612-9242	F0 Female Dry Weight	59	>59	NA	9.8%		Dunnett Multiple Comparison Test
05-3241-8480	F0 Female Dry Weight	59	>59	NA	7.23%		Williams Multiple Comparison Test
04-6548-6527	F0 Female Length	59	>59	NA	3.52%		Dunnett Multiple Comparison Test
07-8686-0057	F0 Female Length	59	>59	NA	2.6%		Williams Multiple Comparison Test
12-7996-7441	F0 Male Dry Weight	59	>59	NA	13.7%		Dunnett Multiple Comparison Test
05-9195-6356	F0 Male Dry Weight	59	>59	NA	10.1%		Williams Multiple Comparison Test
13-2818-5844	F0 Male Length	59	>59	NA	2.42%		Dunnett Multiple Comparison Test
03-2821-8014	F0 Male Length	59	>59	NA	1.79%		Williams Multiple Comparison Test
08-6199-0070	F0 Survival Entire Study	59	>59	NA	35.7%		Dunnett Multiple Comparison Test
14-4988-5739	F0 Survival Entire Study	59	>59	NA	26.3%		Williams Multiple Comparison Test
16-0977-7718	n Offspring Per Female	59	>59	NA	43.9%		Dunnett Multiple Comparison Test
19-8524-4964	n Offspring Per Female	59	>59	NA	32.4%		Williams Multiple Comparison Test
06-6263-7726	Time to First Brood	59	>59	NA	5.91%		Dunnett Multiple Comparison Test
12-8777-4241	Time to First Brood	59	>59	NA	4.36%		Williams Multiple Comparison Test

# CETIS Summary Report

Report Date: 02 Jul-13 10:49 (p 2 of 5)  
 Test Code: 125619 48221501 | 16-2078-2964

OPPTS 850.1350 Chronic Invert (Mysid)

Springborn Smithers

## F0 Female Dry Weight Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	1.03	0.9029	1.157	1.02	1.04	0.01	0.01414	1.37%	0.0%
1.9		2	1.01	0.7559	1.264	0.99	1.03	0.02	0.02828	2.8%	1.94%
4		2	1.05	0.9229	1.177	1.04	1.06	0.01	0.01414	1.35%	-1.94%
7.7		2	1.1	0.8459	1.354	1.08	1.12	0.02	0.02828	2.57%	-6.8%
16		2	1.16	1.033	1.287	1.15	1.17	0.01	0.01414	1.22%	-12.62%
30		2	0.98	0.3447	1.615	0.93	1.03	0.05	0.07071	7.22%	4.85%
59		2	1.08	0.6988	1.461	1.05	1.11	0.03	0.04243	3.93%	-4.85%

## F0 Female Length Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	7.4	7.4	7.4	7.4	7.4	0	0	0.0%	0.0%
1.9		2	7.45	6.815	8.085	7.4	7.5	0.05	0.07071	0.95%	-0.68%
4		2	7.35	6.715	7.985	7.3	7.4	0.05	0.07071	0.96%	0.68%
7.7		2	7.25	6.615	7.885	7.2	7.3	0.05	0.07071	0.98%	2.03%
16		2	7.45	6.815	8.085	7.4	7.5	0.05	0.07071	0.95%	-0.68%
30		2	7.3	6.029	8.571	7.2	7.4	0.1	0.1414	1.94%	1.35%
59		2	7.4	6.129	8.671	7.3	7.5	0.1	0.1414	1.91%	0.0%

## F0 Male Dry Weight Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	0.8	0.1647	1.435	0.75	0.85	0.05	0.07071	8.84%	0.0%
1.9		2	0.785	0.5944	0.9756	0.77	0.8	0.015	0.02121	2.7%	1.88%
4		2	0.705	0.3873	1.023	0.68	0.73	0.025	0.03536	5.02%	11.88%
7.7		2	0.785	0.5944	0.9756	0.77	0.8	0.015	0.02121	2.7%	1.88%
16		2	0.815	0.4973	1.133	0.79	0.84	0.025	0.03536	4.34%	-1.88%
30		2	0.805	0.6144	0.9956	0.79	0.82	0.015	0.02121	2.64%	-0.63%
59		2	0.8	0.4188	1.181	0.77	0.83	0.03	0.04243	5.3%	0.0%

## F0 Male Length Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	6.95	6.315	7.585	6.9	7	0.05	0.07071	1.02%	0.0%
1.9		2	7.05	6.415	7.685	7	7.1	0.05	0.07071	1.0%	-1.44%
4		2	7	7	7	7	7	0	0	0.0%	-0.72%
7.7		2	7.05	6.415	7.685	7	7.1	0.05	0.07071	1.0%	-1.44%
16		2	7.05	6.415	7.685	7	7.1	0.05	0.07071	1.0%	-1.44%
30		2	7	7	7	7	7	0	0	0.0%	-0.72%
59		2	6.85	6.215	7.485	6.8	6.9	0.05	0.07071	1.03%	1.44%

## F0 Survival Entire Study Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	0.7333	0	1	0.6667	0.8	0.06667	0.09428	12.86%	0.0%
1.9		2	0.65	0.01469	1	0.6	0.7	0.05	0.07071	10.88%	11.36%
4		2	0.7333	0	1	0.6	0.8667	0.1333	0.1886	25.71%	0.0%
7.7		2	0.6667	0	1	0.6	0.7333	0.06667	0.09428	14.14%	9.09%
16		2	0.8333	0.4098	1	0.8	0.8667	0.03333	0.04714	5.66%	-13.64%
30		2	0.7667	0.7667	0.7667	0.7667	0.7667	0	0	0.0%	-4.55%
59		2	0.7	0.7	0.7	0.7	0.7	0	0	0.0%	4.55%

## n Offspring Per Female Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	20	20	20	20	20	0	0	0.0%	0.0%
1.9		2	22	9.294	34.71	21	23	1	1.414	6.43%	-10.0%
4		2	20	-30.82	70.82	16	24	4	5.657	28.28%	0.0%
7.7		2	18	5.294	30.71	17	19	1	1.414	7.86%	10.0%
16		2	20	20	20	20	20	0	0	0.0%	0.0%
30		2	16	16	16	16	16	0	0	0.0%	20.0%
59		2	20	-30.82	70.82	16	24	4	5.657	28.28%	0.0%

# CETIS Summary Report

Report Date: 02 Jul-13 10:49 (p 3 of 5)  
Test Code: 125619 48221501 | 16-2078-2964

OPPTS 850.1350 Chronic Invert (Mysid)

Springborn Smithers

## Time to First Brood Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	2	18	18	18	18	18	0	0	0.0%	0.0%
1.9		2	17.5	11.15	23.85	17	18	0.5	0.7071	4.04%	2.78%
4		2	18	18	18	18	18	0	0	0.0%	0.0%
7.7		2	18	18	18	18	18	0	0	0.0%	0.0%
16		2	19	19	19	19	19	0	0	0.0%	-5.56%
30		2	18.5	12.15	24.85	18	19	0.5	0.7071	3.82%	-2.78%
59		2	19	19	19	19	19	0	0	0.0%	-5.56%

# CETIS Summary Report

Report Date: 02 Jul-13 10:49 (p 4 of 5)  
Test Code: 125619 48221501 | 16-2078-2964

OPPTS 850.1350 Chronic Invert (Mysid) Springborn Smithers

F0 Female Dry Weight Detail			
C-µg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	1.04	1.02
1.9		1.03	0.99
4		1.04	1.06
7.7		1.12	1.08
16		1.17	1.15
30		0.93	1.03
59		1.11	1.05

F0 Female Length Detail			
C-µg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	7.4	7.4
1.9		7.5	7.4
4		7.4	7.3
7.7		7.3	7.2
16		7.5	7.4
30		7.2	7.4
59		7.5	7.3

F0 Male Dry Weight Detail			
C-µg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	0.85	0.75
1.9		0.77	0.8
4		0.68	0.73
7.7		0.77	0.8
16		0.79	0.84
30		0.79	0.82
59		0.77	0.83

F0 Male Length Detail			
C-µg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	7	6.9
1.9		7	7.1
4		7	7
7.7		7	7.1
16		7	7.1
30		7	7
59		6.9	6.8

F0 Survival Entire Study Detail			
C-µg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	0.6667	0.8
1.9		0.7	0.6
4		0.8667	0.6
7.7		0.7333	0.6
16		0.8667	0.8
30		0.7667	0.7667
59		0.7	0.7

# CETIS Summary Report

Report Date: 02 Jul-13 10:49 (p 5 of 5)  
Test Code: 125619 48221501 | 16-2078-2964

OPPTS 850.1350 Chronic Invert (Mysid)

Springborn Smithers

## n Offspring Per Female Detail

C-µg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	20	20
1.9		21	23
4		24	16
7.7		19	17
16		20	20
30		16	16
59		16	24

## Time to First Brood Detail

C-µg ai/L	Control Type	Rep 1	Rep 2
0	Negative Control	18	18
1.9		18	17
4		18	18
7.7		18	18
16		19	19
30		18	19
59		19	19